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Development of a new method for the detection and quantification of octopamine and 3-nitrotyrosine

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Introduction: Neurodegenerative diseases affect around 30 million people worldwide, thus new methods that can facilitate research of such diseases are essential. Neurotransmitters, characterised as critical regulators of several neurological disorders, have been the target for the development of such methods. Besides, oxidative/ nitrosative stress has been associated with several neurodegenerative diseases and is thought to play an important role in their pathogenesis. Therefore, the development of bioanalytical methods for quantification of neurotransmitters and oxidative stress-associated molecules seems to be an interesting approach. The aim of this study was to develop a simple, rapid, low-cost and sensitive octopamine (OCT) and 3-nitrotyrosine (3-NT) quantification method for use in biological models.

Materials and Methods: All experiments were performed on a Hitachi LaChrom Elite® HPLC system and detection was accomplished trough a diode array detector. Chromatographic separation was carried out using a Lichrocart® 250-4 Lichrospher 100 RP-18 (5µm) column. A solution of 0.5% CH_3COOH:MeOH:H_2O (15:15:70, v/v) was used as an isocratic mobile phase with a flow rate of 1 mL/min and at 25ºC. The method was developed and validated according to the International Conference on Harmonisation (ICH) guidelines.
Results: The protocol tested showed specificity for both biomolecules at 276 nm. Using this protocol, the calibration curve was linear (correlation coefficient = 1), the limit of detection (LOD) and the limit of quantification (LOQ) were in the order of ng/mL, and the time required for analysis did not exceed 15 minutes per sample.

Conclusion: The proposed method, which was successfully developed for OCT and 3-NT quantification, is simple, cheap and fast. In a near future, it would be interesting to assess whether this method is suitable for the quantification of OCT and 3-NT in several biological models.

Keywords: Octapamine, 3-Nitrotyrosine, HPLC, Neurodegenerative diseases.